Hyperamylasemia, Specific Pancreatic Enzymes, and Hypoxanthine during Recovery from Diabetic Ketoacidosis

Jens Møller-Petersen,1,2 Per T. Andersen,1 Niels Hjørne,2 and Jørn Ditzi1

The contribution of the exocrine pancreas to hyperamylasemia in diabetic ketoacidosis was investigated by measuring total amylase, salivary and pancreatic isoamylases, cathodic trypsin-like immunoreactivity, and pancreatic lipase in 12 consecutive patients recovering from diabetic ketoacidosis. Hyperamylasemia was present in six of the patients [50%; expected incidence: 21.1–78.9% (95% confidence limits)]—in five with simultaneously increased activities of all three specific pancreatic enzymes, and in one with only increased salivary isoamylase. The serum concentration of hypoxanthine—an indicator of the cellular energy state—was above normal in all patients at admission. We found no differences in concentrations of hypoxanthine in serum of patients with or without hyperamylasemia or in patients with or without increases in the specific pancreatic enzymes. In none of the patients was the clinical course or the time-concentration curves of the pancreatic enzymes consistent with acute pancreatitis. The pathogenic mechanism leading to hyperamylasemia in diabetic ketoacidosis remains uncertain.

Additional Keyphrases: acute pancreatitis ∙ amylase isoenzymes ∙ cathodic trypsin-like immunoreactivity ∙ salivary and pancreatic lipase ∙ reference interval

Hyperamylasemia has been reported in 41% to 80% of patients during recovery from diabetic ketoacidosis (Table 1). In three of these reports the concentrations of isoamylases were measured, and in only 14% to 50% of the patients was hyperamylasemia caused by increases in pancreatic isoamylase activity (6–8). This suggests that the exocrine pancreas plays only a minor role in the hyperamylasemia found during diabetic ketoacidosis. Now that methods exist for measuring specific pancreatic enzymes by their antigenic properties and not by their activity in serum (9, 10), we have measured cathodic trypsin-like immunoreactivity, pancreatic lipase, and pancreatic isoamylase in patients recovering from diabetic ketoacidosis—using these new methods to reassess the contribution to hyperamylasemia by the exocrine pancreas. In an attempt further to elucidate the pathogenetic mechanisms of hyperamylasemia in these patients, we have also measured the serum concentration of hypoxanthine, a purine base the presence of which is correlated inversely to the status of intracellular energy (11–13).

Patients and Methods

Twelve patients, three women and nine men, admitted consecutively to hospital with diabetic ketoacidosis, were investigated from admission to full recovery. The diagnosis of diabetic ketoacidosis was based on excessive ketonuria, a bicarbonate concentration in plasma <18 mmol/L, and an arterIALIZED capillary pH <7.35.

The treatment, a "low-dose insulin" schedule, provided 12 units of crystalline insulin intravenously per hour and intravenous fluid replacement with isotonic saline, potassium chloride, and additional glucose when glycaemia was <10 mmol/L. No patient received sodium bicarbonate or phosphate supplement. Blood samples were taken at admission, every 4 h for the first 24 h, and thereafter at the second day, third day, and seventh day of hospitalization.

The concentrations of plasma bicarbonate and serum phosphate, and the arterialized capillary pH were measured by routine laboratory methods. Creatinine concentrations in serum were measured by a specific creatinin method (14) and glucose in capillary blood by an AutoAnalyzer (15). Concentrations of β2-microglobulin in serum were determined with a double-antibody radioimmunoassay (Beta-2-micro RIA; Pharmacia Diagnostics AB, Uppsala, Sweden). Ketone bodies in urine were determined by using test strips (Glukestur-Test; Boehringer Mannheim GmbH, Mannheim, F.R.G.).

Table 2 summarizes the laboratory data for the 12 patients at admission. Seven patients—four with increased pancreatic enzymes and three with normal activity concentrations of the enzymes—had upper abdominal pain at admission, which disappeared after 8 h of the treatment detailed above.

Concentrations of cathodic trypsin-like immunoreactivity in serum was measured with a double-antibody radioimmunoassay (RIA-gnost·Trypsin), whereas pancreatic lipase was measured with an enzyme immunoassay (Enzygnost·Lipase), both from Behringwerke, Marburg/Lahn, F.R.G. To measure the concentrations in serum of total amylase and salivary and pancreatic isoamylases, we used a photometric centrifugal analyzer (Cobas-Bio®, Hoffmann-La Roche, Basel, Switzerland), and a wheat-inhibitor method (Phadebas·Kinetic Amylase with Phadebas KAI reagents; Pharmacia Diagnostics AB). The serum concentration of hypoxanthine was measured with a xanthine oxidase method (16).

Reference intervals. Reference intervals for total amylase and all three specific pancreatic enzymes were established in 60 healthy subjects (median age 35 years, range 21–64) and in 50 nonketotic insulin-dependent diabetics (median age 31 years, range 18–50 years). We used a method described by Massod (17) and report the respective upper limits of these reference intervals for healthy subjects and for nonketotic diabetics in Figures 1–3. The reference interval for hypoxanthine in both healthy subjects and insulin-dependent diabetics was 0–8 μmol/L.

Statistical methods. We used nonparametric methods, comparing two groups by the Mann–Whitney test. For analysis of variance in a variable over time we used Friedman's test as modified by Conover (18) for pairwise comparison if an overall difference was found. The Spearman rank correlation coefficient (ρ) was used to measure the association between two variables. The 95% confidence limits for a given proportion were calculated from tables and formulas (19) on the assumption of a binomial distribution. A 5% (two-tailed) level of statistical significance was used.

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Received July 22, 1985; accepted September 6, 1985.
Table 1. Number and Relative Frequency of Patients with Increased Activities of Total Amylase and Isoamylases in Serum during Diabetic Ketoacidosis, as Reported Previously

<table>
<thead>
<tr>
<th>Ref.</th>
<th>N</th>
<th>%</th>
<th>Total amylase N</th>
<th>Pancreatic isoamylase N</th>
<th>Salivary isoamylase N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8/11*</td>
<td>72.7 (39.0–94.0)</td>
<td>1/7 (0.4–57.9)</td>
<td>6/7 (42.1–99.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11/27</td>
<td>40.7 (22.3–61.2)</td>
<td>25 (27.0–68.7)</td>
<td>9/25 (18.0–57.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21/35c</td>
<td>60.0 (42.1–76.1)</td>
<td>12/25c (0.4–57.9)</td>
<td>9/25c (18.0–57.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8/10</td>
<td>80.0 (44.9–97.5)</td>
<td>2/4 (50.0–63.9)</td>
<td>9/25 (18.0–57.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>9/14</td>
<td>64.3 (35.1–87.2)</td>
<td>2/4 (50.0–57.9)</td>
<td>9/25 (18.0–57.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7/13</td>
<td>53.8 (25.1–77.0)</td>
<td>2/4 (50.0–63.9)</td>
<td>9/25 (18.0–57.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>26/33d</td>
<td>78.8 (61.1–91.0)</td>
<td>12/25 (0.4–57.9)</td>
<td>9/25 (18.0–57.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4/9</td>
<td>44.4 (13.7–78.8)</td>
<td>2/4 (50.0–57.9)</td>
<td>9/25 (18.0–57.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94/152</td>
<td>61.8 (54.9–68.4)</td>
<td>15.36 (25.5–59.2)</td>
<td>17/36 (30.4–67.1)</td>
<td></td>
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</tr>
</tbody>
</table>

*No. of patients with increased enzyme activity/no. of patients with diabetic ketoacidosis. **95% confidence limits listed in parentheses. **35 episodes of diabetic ketoacidosis in 29 patients. **33 episodes of diabetic ketoacidosis in 31 patients. **Isoamylase analyses were done in 25 of the 26 patients with hyperamylasemia. In four patients the hyperamylasemia was due to equal increases in pancreatic and salivary isoamylase activity.

Table 2. Laboratory Data for 12 Patients with Diabetic Ketoacidosis at Admission

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Median (and range)</th>
<th>Reference intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-glucose, mmol/L</td>
<td>32.0 (15.2–51.2)</td>
<td>3.1–5.6</td>
</tr>
<tr>
<td>P-bicarbonate, mmol/L</td>
<td>9.9 (8.0–16.3)</td>
<td>21.3–25.8</td>
</tr>
<tr>
<td>Arterialized capillary pH</td>
<td>7.17 (7.00–7.32)</td>
<td>7.35–7.42</td>
</tr>
<tr>
<td>S-creatinine, μmol/L</td>
<td>96 (62–217)</td>
<td>55–125</td>
</tr>
<tr>
<td>S-β₂-microglobulin, nmol/L</td>
<td>178 (93–686)</td>
<td>70–120</td>
</tr>
<tr>
<td>S-phosphate, mmol/L</td>
<td>1.42 (0.64–3.60)</td>
<td>0.80–1.55</td>
</tr>
<tr>
<td>S-osmolality, mosm/kg H₂O</td>
<td>337 (302–382)</td>
<td>280–290</td>
</tr>
<tr>
<td>U-ketone bodies, mmol/L</td>
<td>&gt;10 (all patients)</td>
<td>&lt; 0.5</td>
</tr>
</tbody>
</table>

*Measured semiquantitatively with test strips: B: blood; P: plasma; S: serum; U: urine.

Results

Figure 1 shows the changes of serum total amylase in two groups of patients during recovery from diabetic ketoacidosis. Six patients (50%; 95% confidence limits: 21.2–78.9%) had hyperamylasemia (amylase exceeding the reference interval for healthy subjects) either at admission to hospital or during recovery (p < 0.01, Friedman test). In the remaining six patients serum amylase was initially low with slightly increasing values (p < 0.01, Friedman test) during recovery. There was a statistically significant difference between the two groups (p < 0.01, Mann–Whitney test) within the first 24 h and on day 2 for total serum amylase. The hyperamylasemia was due to increased salivary isoamylase in one patient and to increased pancreatic isoamylase in five patients.

Figure 2 shows the changes in pancreatic isoamylase, cathodic trypsin-like immunoreactivity, and pancreatic lipase at admission and during recovery. There was a simultaneous increase of all three specific pancreatic enzymes in five patients (41.7%; 95% confidence limits: 15.2–72.3%). For all three enzymes the change in activity with time was statistically significant for the patients with increased enzymes (group 1) as well as for those (group 2) with normal or low enzyme concentrations (p < 0.01, Friedman test). Within the first 24 h and on day 2, there was a statistically significant difference between group 1 and group 2 (p < 0.01, Mann–Whitney test) for all three enzymes. One patient in group 1 still had a persistent increase of all three enzymes on day 7. This patient also had increased concentrations of creatinine and β₂-microglobulin in his serum on day 7—an indication that decreased renal function was a probable cause of the persistently increased enzyme activities.

Between patients with or without hyperamylasemia or between patients with or without increases in specific pancreatic enzymes we found no differences regarding: age, duration of diabetes mellitus, fluid deficit, insulin requirement, degree of acidosis, glucose concentration in blood, serum osmolality, or renal function (as estimated from results for serum creatinine and β₂-microglobulin). Concentrations of serum phosphate at 8 and 12 h after admission were lower in patients with increased pancreatic enzymes than in patients with normal enzyme activities (p < 0.01, Mann–Whitney test). However, in only one patient (who had normal enzyme concentrations) was there a statistically significant correlation (Spearman’s r) between serum phosphate and any of the three specific pancreatic enzymes or total amylase.

Figure 3 shows the changes of concentrations of hypoxanthine in serum during recovery from diabetic ketoacidosis. In all patients this analyte was increased at admission and gradually decreased during recovery (p < 0.01, Friedman test). We found no difference between the two groups with respect to the hypoxanthine concentration.

Discussion

In this study we found hyperamylasemia in six of 12 patients recovering from diabetic ketoacidosis. Five had
Our results suggest that the exocrine pancreas is a major contributor to the increased amylase activity in serum of patients recovering from diabetic ketoacidosis. However, some patients may show normal concentrations of specific pancreatic enzymes with a simultaneous increase of salivary isoamylase. The part played by the exocrine pancreas with respect to hyperamylasemia seems to be larger in our study than reported by others (6-8, 20).

Decreases in inorganic phosphate in plasma during therapy with insulin have previously been correlated to hyperamylasemia in diabetic ketoacidosis (7, 8). These authors speculated that an increased oxygen affinity to hemoglobin, induced by decreasing the inorganic phosphate in plasma, combined with reduced splanchnic perfusion could prevent the generation of ATP and damage pancreatic tissue. When ATP generation decreases, high-energy phosphate degradation increases (13). Consequently we measured the concentration of hypoxanthine, a degradation product of nucleotides (primarily ATP), in serum. Because no difference could be demonstrated between patients with normoamylasemia and hyperamylasemia, we found no support for the above hypothesis.

One could argue that the increase of specific pancreatic enzymes in our patients was caused by acute pancreatitis, the only adequate diagnostic "gold standard" for which is pathoanatomical examination of pancreatic tissue. Because none of our patients died or underwent abdominal surgery, we had to rely on the clinical course as a diagnostic key. In acute pancreatitis the serum concentrations of cathodic trypsin-like immunoreactivity, pancreatic lipase, and pancreatic isoamylase are increased for a more prolonged period (several days) (21-24) than we saw in our patients; thus, it seems unlikely that any of our patients had acute pancreatitis.

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**Fig. 2.** Changes in concentrations of pancreatic enzymes in serum during diabetic ketoacidosis

Broken and solid lines and horizontal bars denote same parameters as in Fig. 1. (○), patients with increased enzyme activity at admission or during recovery, exceeding the upper reference limit in healthy subjects; (□), patients without such an increase. Top, pancreatic isoamylase; middle, cathodic trypsin-like immunoreactivity; bottom, pancreatic lipase.

**Fig. 3.** Changes in concentrations of hypoxanthine in serum during diabetic ketoacidosis

Broken line marks upper reference limit in both healthy subjects and nonketotic insulin-dependent diabetics. (○), patients with increased total amylase due to increased salivary isoamylase; (□), patients without an increase of any enzyme; (●), patients with increased total amylase and specific pancreatic enzymes. Horizontal bars indicate median hypoxanthine values for all 12 patients.
In conclusion, 50% of our patients recovering from diabetic ketoacidosis had hyperamylasemia and 42% had increased activities of specific pancreatic enzymes. None had a clinical course compatible with acute pancreatitis. The pathogenetic mechanism leading to increased concentrations of pancreatic enzymes in the serum of these patients remains obscure.

Financial support was received from the North Jutland Research Fund and from the Research Fund of Aalborg City Council.

References